THE EFFECTS OF VITAMIN E DEFICIENCY ON THE TADPOLE OF *RANA PIPIENS*

BY

EDNA MAE PRATT

B.S., University of Illinois, 1936

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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY
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THE EFFECTS OF VITAMIN E DEFICIENCY ON THE TADPOLE OF *RANA PIPIENS*

The tadpoles used in this study were reared in the laboratory from eggs. One group of tadpoles was hatched from eggs gathered from a temporary pond on March 30, 1938, near Urbana, Illinois. The other two groups were from eggs obtained by injecting adult male and female *Rana pipiens* frogs with pituitary glands from other adult frogs of the same species. In this way ovulation and fertilization were caused to take place out of the regular breeding season, one mass of eggs being obtained October 1, 1937, and the other mass November 17, 1938.

The tadpoles were divided into series according to the treatment they received. Series A and B received a ration composed of ground yellow corn, 46 parts; wheat bran, 13 parts; wheat middlings, 13 parts; alfalfa meal, 5 parts; meat scrap, 10 parts; dried skim milk, 10 parts; salt, 1 part; and sardine oil, 2 parts. To remove vitamin E from this ration, the food was treated with a one per cent solution of ferric chloride dissolved in ether. After soaking for 12 hours, the ether was distilled off and the sardine oil was added to offset any destruction of vitamin A which is oxidized by ferrous salts.

Series C received a synthetic vitamin E-deficient ration composed of casein, 25%; corn starch, 54%; lard, 15%; salt mixture, 4%; yeast, 1.5%; and cod-liver oil, 0.5%.

Series D received the same ration as A and B, untreated with ferric chloride. This series contained the normal controls.

Series E received thyroid substance to see if metamorphosis could be induced in the E-free tadpoles.

Series F received anterior pituitary substance in another attempt to induce metamorphosis in the E-free tadpoles.

Series G received pituitrin injections to see its effect in relation to pigmentary changes occurring in the E-deficient tadpoles.

Series H received wheat germ oil in an attempt to repair any of the E-deficiency effects.

The organs sectioned and examined were the anterior lobe of the pituitary gland stained with Clements' modification of Mallory's triple, the thyroid gland stained with Mallory's triple, and the gonads stained with Heidenhain's iron alum haematoxylin. Total mounts were prepared of skin taken from the backs

and tails of the specimens.

A relative vitamin E deficiency is produced in the tadpole of *Rana pipiens* by feeding a ferric chloride treated ration or a synthetic vitamin E-free ration. The effects of vitamin E deficiency on the tadpole are as striking as those observed in some of the higher vertebrates suffering from a deficiency in this vitamin. The lack of vitamin E in the tadpole results in: 1. Failure to undergo metamorphosis, 2. a tendency toward albinism, 3. distention of the body cavity with fluid along with atrophy of the digestive tract, 4. no alteration in growth rate, a steady increase in size occurring with the increase in age, 5. no atrophy or retardation in the development of the gonads and no degeneration of the germinal epithelium of the testis. It must be stressed that it is only occasionally that the first three of these effects are found in the same individual. Frequently the first two effects are found in the same animal, although only the first, without the second, may be observed at times. The third condition is usually found in the younger animals and results in death after a short time.

Out of about 77 tadpoles fed with a vitamin E-deficient ration, only eight individuals underwent metamorphosis. It is believed that there was a sufficient amount of vitamin E in the eggs from which they developed to protect them from the effects of the diet, thus allowing them to undergo metamorphosis.

After reviewing the literature on tadpole endocrine glands, it was found that vitamin E-deficient tadpoles which failed to metamorphose exhibited all the characteristics found in thyroidectomized tadpoles. This suggested inactivity of the thyroid gland. Thyroid feeding and thyroid injection successfully induced metamorphosis in the experimental animals. The possibility that the thyrotropic hormone of the anterior pituitary might be affected was likewise taken into consideration. Metamorphosis was successfully produced in the experimental animals with injections of anterior lobe substance. Thus it appeared that there was non-function of the thyrotropic hormone of the anterior lobe of the pituitary gland and inactivity of the thyroid gland, the combined effect of which resulted in the failure of the vitamin E-deficient tadpoles to undergo metamorphosis. In one case definite signs of metamorphosis were produced by

an injection of wheat germ oil. This seemed to indicate that the anterior lobe of the pituitary and the thyroid gland had been merely inactivated by the lack of vitamin E in the diet.

Histologically the anterior lobe of the pituitary gland of the experimental animals showed no particularly significant effects because of the uncertainty of the staining reactions. Histologically the thyroid gland was found to show great variability, not only between individual specimens but within the same individual. Due to the fact that flattened, cubical, or columnar epithelium, and follicles filled with non-vacuolated colloid, contracted colloid or no colloid were found in the thyroid glands of the E-deficient specimens, the views of other investigators who claimed that columnar epithelium and vacuolated colloid were signs of activity of the thyroid gland were not accepted in this investigation. Instead the observations of Etkin that increase in cell number and colloid volume in the entire gland are indications of metamorphosis was accepted because it was noted that the gland of the experimental animals was larval in configuration and consequently contained fewer follicles with a smaller number of cells and lesser amount of colloid than occurred in animals undergoing metamorphosis.

The investigation of the pale pigmentation of some of the vitamin E-deficient animals showed that a reduction in the number and melanin content of the epidermal melanophores of the skin occurred. Also there was a great reduction in the amount of free epidermal pigment and a partial or complete expansion of the xantholeucophores with an apparent reduction in the number of these cells. Upon review of the literature, a comparable condition was found to result from hypophysectomy either of the entire hypophysis or of just the intermediate lobe. More recent literature showed that pigmentation in the frog is apparently controlled by the secretion of the intermediate lobe of the pituitary. Thus, it seemed apparent that the lack of vitamin E had also inactivated the intermediate lobe in the experimental animals. Injections of post-pituitary substance caused darkening of the animals and, upon examination of the skin, it was found that the number and melanin content of the epidermal melanophores had been restored to approximately the normal condition, and there was a partial restoration of the free epidermal

pigment. Conversely to Smith's observations, however, the xantholeucophores were contracted after post-pituitary treatment.

Growth in the vitamin E-deficient tadpoles was apparently not affected, a steady increase in size occurring with the increase in age. It seemed apparent that the growth-promoting hormone of the anterior pituitary was functioning normally during the prolonged larval existence of these animals. The fact that vitamin E may have a growth-promoting effect was taken into consideration and the conclusion reached that in the tadpole growth is apparently not entirely dependent upon the presence of this vitamin in the diet.

The gonads of the vitamin E-deficient tadpoles were found to develop normally, showing no signs of atrophy or retardation which would indicate a disturbance of the gonad-stimulating hormone of the hypophysis. The condition, instead, resembled that occurring in thyroidectomized tadpoles undergoing a prolonged larval existence. In the older female E-free tadpoles the oocytes became extremely large, and in the older male E-free animals the testes were found to contain mature spermatozoa. In the male there were no signs of the degeneration of the germ cells of the testes which occurs in vitamin E-deficient higher vertebrates. It appears that the testes of the tadpoles of *Rana pipiens* are able to resist this degeneration caused by the lack of vitamin E in the diet during an extended larval period.

Sometimes the experimental animals showed a condition in which the body cavity became distended with fluid and the digestive organs underwent atrophy. The cause of this condition was not determined, but it is suggested that it may possibly be related to an upset in the regulation of water metabolism by the posterior lobe of the pituitary gland. Since various investigators have shown that injections of pitressin cause a change in skin permeability in frogs with an increase in water uptake, it is thought that in the E-deficient tadpoles a similar change may occur due to overactivity of the posterior lobe of the pituitary so that a greater water intake occurs.

The atrophy of the digestive tract may be caused by starvation due to the failure of the animals to eat. The loss of appetite may be due to an upset of the whole body metabolism from abnormal functioning of the endocrine system.

VITA

Edna Mae Pratt was born in Champaign, Illinois, March 28, 1914, and attended the elementary schools of that city. She was graduated from Champaign High School in 1932. Her undergraduate education was obtained at the University of Illinois, where she received the Bachelor of Science degree in June 1936, graduating with high honors in Zoology. She entered the Graduate School at the same institution after receiving a Service Scholarship and obtained the Master of Science degree in June 1937. The summer of 1937 was spent as an assistant in Zoology. She received a Fellowship in Zoology for the academic year of 1937-1938 and the third-year Fellowship for 1938-1939. The summer of 1938 was spent in marine study at the Biological Laboratory located at Cold Spring Harbor, Long Island, New York. During the year 1939-1940 she held a research assistantship in Zoology.

She is a member of Phi Beta Kappa, Sigma Delta Epsilon, Phi Sigma, Phi Kappa Phi, Sigma Xi, and the American Association for the Advancement of Science.

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